Application of baking yeast to induce rooting in hardwood cuttings of olive (*Olea europaea* L.) cv. Sorani

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Abstract

This research was conducted in the open environment at the University of Sulaimani, College of Agricultural Engineering Sciences, to study the effect of different concentrations of baker's yeast and soaking time on the rooting of hardwood cuttings of olive *Olea europaea* L. cv. Sorani. The hardwood cuttings of olive were soaked in (1, 2, 3 and 4 g.L$^-$) yeast concentrations for two different soaking times (1 hr. or 2 hrs.). The cuttings soaked in 2 g.L$^-$ yeast gave the highest values of rooting percentage (43.33%), root number (3.02), root length (12.78 cm), shoot diameter (2.19 mm), sprout bud number (4.27) and IAA content (123.61 µg.mL$^-$). Inversely, the soaking time had no significant effects on studied parameters except IAA content which gave the higher significant value (119.01 µg.mL$^-$) in cuttings soaked in yeast concentrations for 1 hour. As for interaction of yeast concentrations with soaking times, the cuttings soaked in 2 g.L$^-$ yeast for 2 hours showed the maximum rooting percentage (46.66%) and root length (14.02 cm), while interaction of the same yeast concentration with 1 hour soaking gave the higher means of number of roots (4.05), number of sprout bud (4.33) and IAA content (145.37 µg.mL$^-$). Moreover, shoot diameter gave the highest mean (2.46 mm) due to the interaction of 4 g.L$^-$ yeast with 1hour soaking.

Keywords: ***Olive cuttings, rooting percentage, yeast concentration, soaking time.***

Introduction

Seeking for synthetic growth regulator alternatives has become the case of many studies carried out by many researchers and propagators, when used as rooting promoters in cuttings and other propagules. Synthetic growth regulators are expensive, and propagation of agricultural cultivars under the effect of synthetic growth regulators is not recommended in organic agriculture [3]. Besides, using natural sources of growth regulators in agriculture protects human and environment form adverse effects of artificial plant growth regulators in growing plants [1]. Many natural materials contain auxin and other substances which could be applied instead of synthetic growth regulators in cutting propagation [6].

Yeast (*Saccharomyces cerevisiae*) has been used in many researches as a substitute for improving vegetative growth and increasing yield in agricultural crops which are rich in phytohormones especially cytokinins, vitamins, enzymes, amino acids and minerals, and application for plants increased cell division, protein content, nucleic acid synthesis and chlorophyll formation [2]. Furthermore, spraying yeast on *faba* bean increased endogenous hormones (auxins and cytokinins), while decreased abscisic acid [11]. Yeast extract is used in tissue culture media as a source for vitamins and amino acids such as glycine, lysine and arginine, and induced direct formation of adventitious embryos in some plant species [14]. Additionally, yeast extract acted as a nutrient source and an elicitor in *Glehnia littoralis* culture, thus had positive effect on root growth [10].

For propagation of olive by vegetative method, hardwood and semi-hardwood cuttings are

mostly used. Despite, ability of olive for rooting and quality of roots are low, especially in difficult-to-root cultivars [23]. But, [22] concluded that rooting of olive cuttings increased under the effect of higher hormone concentrations. In the light of these findings, this research is selected to study the effect of different concentrations of yeast on hardwood cuttings of olive as dipped for different times.

Materials and methods

This research was conducted in the open environment at the University of Sulaimani, College of Agricultural Engineering Sciences, to study the effect of different concentrations of baker's yeast and soaking time on the rooting of hardwood cuttings of olive *Olea europaea* L. cv. Sorani.

Hardwood cuttings were taken from one-year-old branches of olive trees on March 11, 2019 with 20 cm length and 0.5-0.8 cm in diameter, then the cutting bases were dipped in different yeast concentrations (1, 2, 3 and 4 $g.L^{-}$) for two different times (1 hr. or 2 hrs.). The concentrations of yeast were previously prepared by dissolving the yeast in distilled water and stirred for 20 minutes at 35 °C, afterwards, the cuttings were immediately placed into the yeast solutions for either 1 or 2 hr. Finally, the cutting bases were treated with 1:8 (50%) Captan fungicide in talc powder, and stuck in sterilized sand medium put in black plastic bags. At the beginning, the cutting were covered by plastic sheet for 25 days to protect them from low temperatures, following this period, the plastic cover sheets were removed, but after 2 months the cuttings were covered again by green shade net (Saran) to protect against intense sunlight.

Estimation of IAA content

After 30 days of planting the cuttings, from the replications of each treatment, one cutting was collected for estimation of IAA content in the cuttings. The estimation was conducted according to [9], in which the samples (the base part of the cuttings) were pulverized in liquid nitrogen, then 1g of the sample extracted with 5.0 ml of 35% perchloric acid, and another 1g with 5.0 ml of Salkowski reagent, after leaving for 1 hour in the dark, the samples were centrifuged at 5000 rpm for 30 min. The supernatant was collected and used for spectrophotometric reading at 530 *nm*. For the same treatment, reading of perchloric acid extraction was subtracted from Salkowski reagent extraction, and then the concentration of IAA was found using the indole-3-acetic acid (IAA) standard curve. The IAA concentrations for preparing the standard curve were also adjusted by subtracting spectrophotometric reading of an IAA concentration in 5.0 ml of 35% perchloric acid from the same, equivalent IAA concentration in 5.0 ml of Salkowski reagent.

Statistical analysis

The experimental design was laid out in a randomize complete block design (RCBD) with three replications, in each replication, 6 cuttings were used in one plastic bag. After 6 months, on September 11, 2019, the data were collected, rooting percentage and subsequent rooting parameters such as root numbers, length of the longest root, shoot length, shoot diameter and number of sprout buds were taken. The data were analyzed by using XLSTAT computer program, and Dancun's multiple range test ($P \leq 0.05$) was used for compering of the means.

Results and discussion

The results shown in (Table 1) demonstrate that yeast concentrations were significantly effective in improving rooting percentage of hardwood cuttings of olive cv. Sorani. Soaking cuttings in 2 $g.L^{-}$ yeast significantly increased rooting percentage (43.33%) compared to control cuttings which gave the lowest rooting (13.33%). Besides, no differences in rooting percentage were recorded among cuttings soaked in 1, 3 or 4 $g.L^{-}$ and control cuttings. The same table shows that root number was the

highest (3.02) in cuttings soaked in 2 g.L^{-} yeast, and they were significantly different from control cuttings and those soaked in 1 g.L^{-} yeast, the control cuttings gave the smallest (1) number of roots. For root length parameter, it was revealed that 2 g.L^{-} yeast produced the significant longest root (12.78 cm), inversely the shortest root (1.08 cm) was observed in cuttings soaked in 1 g.L^{-} yeast. Additionally, the effect of yeast concentrations on shoot traits gave significant results for shoot diameter and sprout bud number, but they did not produce different shoot length significantly. Shoot diameter of the cuttings soaked in 2 g.L^{-} yeast were shown to be the thickest shoots (2.19 mm), however the thinnest shoots (0.68 mm) were observed in the cuttings soaked in 1 g.L^{-} yeast, followed by control cuttings (0.94 mm). Soaking cuttings in 2 g.L^{-} yeast induced the buds to sprout in the highest number (4.27), in opposite cuttings soaked in 1 g.L^{-} yeast resulted in the minimum number of sprouted buds (1.00). It is interesting to note that 2 g.L^{-} yeast enhanced rooting percentage and other studied parameters, these may be due to that yeast contains many biochemical compounds which may have significant consequences on rooting behaver of olive cv. Sorani cuttings. Amino acids, including aspartic and glutamic acids, glycine, tyrosine, and tryptophan, are components of yeast which may have roles in adventitious root formation when they were applied exogenously to the cuttings [15; 4; 17]. In this regard, [8] argued that rooting of *Passiflora actinia* Hook stem was enhanced as a result of seaweed extract application, because of seaweed extract is rich in amino acids. In addition, cytokinin is a growth regulator in plants also exists in yeast, and role of cytokinin in root initiation and development in cuttings is variable depending on its concentration. High concentration of cytokinin restrains root initiation in cuttings, whereas it sufficiently improves root length and lateral root number, additionally cytokinin at low concentrations may enhance adventitious root formation as well [20; 5; 18].

On the other hand, the effect of soaking periods of the cuttings in different concentrations of baking yeast on root and shoot traits were not different statistically for any studied traits (Table 1). These can be explained by that, increasing the soaking period from 1 to 2 hours may not be enough to produce differences among measured parameters, and more periods of soaking than 1 and 2 hours in yeast concentrations were likely required for indicating soaking period effect.

Table 1. Individual effects of yeast concentration (g.L^{-}) and Soaking time (hr) on rooting%, root and shoot traits of hardwood cuttings of olive cv. Sorani.

Treatments	Rooting%	Root No.	Root length (cm)	Shoot length (cm)	Shoot diameter (mm)	Sprout bud number
Yeast concentration (g.L^{-})						
0	13.33 b	1 b	2.55 bc	1.13 a	0.94 bc	1.66 ab
1	16.66 b	1.16 b	1.08 c	1.03 a	0.68 c	1 b
2	43.33 a	3.02 a	12.78 a	3 a	2.19 a	4.27 a
3	33.33 ab	2.58 ab	7.70 ab	3.12 a	1.87 ab	3.75 a
4	20 b	1.96 ab	5.45 bc	2.55 a	2.03 ab	2.50 ab
Soaking time (hr)						
1	26.66 a	2.80 a	6.11 a	2.05 a	1.78 a	3.08 a
2	30 a	1.56 a	7.39 a	2.79 a	1.61 a	2.68 a

* The values in each column with the same letter do not differ significantly according to Duncan's Multiple Range Test ($P \leq 0.05$).

Interaction of the two factors, yeast concentrations and soaking periods, which are shown in (Table 2) exhibited that soaking hardwood cuttings of olive cv. Sorani in 2 g.L^{-} yeast for 2 hours significantly improved rooting percentage over control cuttings and cuttings soaked in 1 g.L^{-} and 4 g.L^{-} yeast for both periods 1 and 2 hours as well. Rooting percentage reached the highest value (46.66%) in cuttings soaked in 2 g.L^{-} yeast for 2 hours, while rooting percentage was reduced to the lowest value (13.33%) in control cuttings and also cuttings soaked in 1 g.L^{-} yeast for 1 hour. Comparison of root number means disclosed that cuttings soaked in 2 g.L^{-} yeast for 1 hour significantly resulted in the best root number (4.05), while root number was reduced to the lowest (0.93) in cuttings soaked in 4 g.L^{-} yeast for 2 hours, control cuttings (1.00) and cuttings soaked in 1 g.L^{-} for 2 and 1 hr (1.00 and 1.33, respectively). Length of the roots were significantly increased to the longest (14.02 cm) in cuttings soaked in 2 g.L^{-} yeast for 2 hours, whereas the shortest root (0.06 cm) was recorded in cuttings soaked in 1 g.L^{-} yeast for 2 hours. Besides, shoot traits data showed that the interaction of the two factors did not significantly affect shoot length, however their interactions were significant for shoot diameter and sprout bud number. The thickness of sprout shoots of the cuttings soaked in 4 g.L^{-} yeast for 1 hour was the highest (2.46 mm), while it was thinned to the lowest value (0.59 mm) by soaking the cuttings in 1 g.L^{-} yeast for 1 hour. Soaking cuttings in 2 g.L^{-} for 1 hour induced more buds to sprout (4.33), however the minimum number of buds sprouted (0.33) was noticed in the cuttings soaked in 1 g.L^{-} yeast for 2 hours. It is worthy to mention that increasing soaking period of the cuttings from 1 to 2 hours in the same concentration did not significantly improve the studied parameters. The differences in measured parameters among cuttings under the effect of interaction of the two factors may mostly belong to the concentrations of yeast rather than the period of soaking in these concentrations.

Table 2. Interaction effect of yeast concentration (g.L^{-}) and soaking time (hr) on rooting%, root and shoot traits of hardwood cuttings of olive cv. Sorani.

Yeast concentration (g.L^{-})	Soaking time (hr)	Rooting%	Root No.	Root length (cm)	Shoot length (cm)	Shoot diameter (mm)	Sprout bud Number
tim0	0 hr	13.33 c	1 b	2.55 c	1.13 a	0.94 bcd	1.66 ab
1	1 hr	20 bc	1.33 b	2.10 c	1.06 a	0.59 d	1.66 ab
	2 hrs	13.33 c	1 b	0.06 c	1 a	0.78 cd	0.33 b
2	1 hr	40 ab	4.05 a	11.55 ab	2.63 a	2.14 abc	4.33 a
	2 hrs	46.66 a	2 ab	14.02 a	3.37 a	2.24 ab	4.22 a
3	1 hr	33.33 abc	2.83 ab	3.21 bc	2.30 a	1.93 abcd	3.33 ab
	2 hrs	33.33 abc	2.33 ab	12.18 ab	3.95 a	1.81 abcd	4.16 a
4	1 hr	20 bc	3 ab	7.60 abc	2.30 a	2.46 a	3 ab
	2 hrs	20 bc	0.93 b	3.30 bc	2.80 a	1.61 abcd	2 ab

* The values in each column with the same letter do not differ significantly according to Duncan's Multiple Range Test ($P \leq 0.05$).

The data illustrated in (Figure 1) explain that yeast concentrations significantly improved indole-3-acetic acid (IAA) content in the cuttings. The cuttings soaked in 2 g.L^{-} yeast were significantly different with the other concentrations. The highest IAA content (123.61 µg.ml^{-}) was found in the cuttings soaked in 2 g.L^{-} yeast, whereas the lowest IAA content (74.22 µg.ml^{-}) occurred in the control cuttings (Figure 1A). At the same time, the effect of soaking time of the cuttings in yeast concentrations on IAA content in the cuttings were significant as well (Figure 1B). The cuttings which had been soaked in yeast concentrations for 1 hour produced more IAA than those soaked for 2 hours, soaking the

cuttings for 1 hour gave (119.01 µg.ml⁻) IAA content, whereas soaking the cuttings for 2 hours showed (91.98 µg.ml⁻) IAA content. Moreover, Interaction of yeast concentrations with soaking times enhanced IAA content in the cuttings significantly (Figure 1C). The cuttings soaked in 2 g.L⁻ and to the lesser extent 3 g.L⁻ yeast for 1 hour significantly produced the highest IAA contents (145.37 and 128.15 µg.ml⁻, respectively) in comparison to control cuttings and those soaked in the rest of yeast concentrations for different times. In contrast, the cuttings soaked in 1 g.L⁻ yeast for 2 hours followed the control cuttings demonstrating the lowest IAA contents (70.21 and 74.22 µg.ml⁻, respectively).

Improving IAA contents in the cuttings as a result of application of yeast concentrations for 1 and 2 hours may be due to that yeast contains many amino acids including tryptophan, as was mentioned previously, and the results of exogenous application of tryptophan to the plants confirmed to have a role in increasing IAA contents because tryptophan is converted to IAA, and the IAA content ratio changed depending on tryptophan concentrations [16; 13; 19]. Similarly, IAA is one of the hormones that increased to the highest level in leaves of snap bean when it was sprayed by yeast [7]. On the other hand, yeast contains many minerals that participate in IAA metabolism and formation either directly or indirectly, such as Zn and Mn [12]. Zinc plays a part in biosynthesis of main auxin precursor amino acid tryptophan, and zinc is a component of Auxin-Binding Protein 1 (ABP 1). As for manganese, it is one of structural constituents of peroxidase enzyme which can influence on IAA metabolism [21].

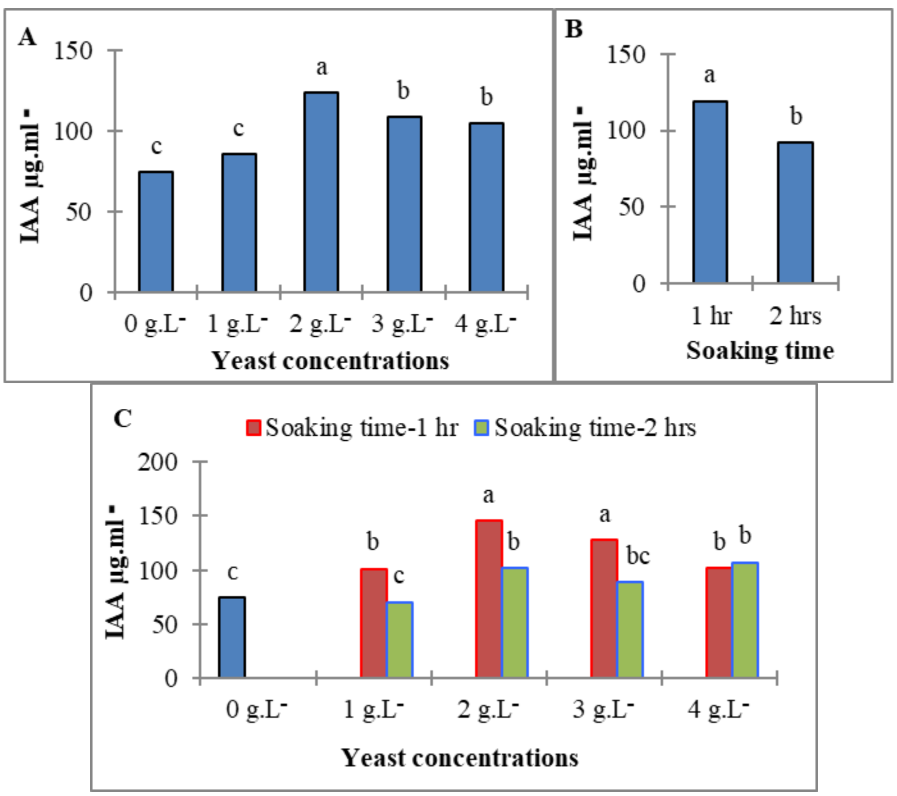

Figure 1. IAA content in olive *Olea europaea* L. cv. Sorani hardwood cuttings after 30 days of planting the cuttings, (A) effect of yeast concentrations, (B) effect of soaking time in yeast concentrations, (C) Interaction effects of yeast concentrations with soaking times. The values on each column with the same letter do not differ significantly according to Duncan's Multiple Range Test (*P*≤0.05).

Conclusion

The data of this experiment cleared that 2 g.L^{-} yeast in which hardwood cuttings of olive cv. Sorani were soaked gave the best rooting percentage, plus other root and shoot traits and also, the most importantly, IAA contents in the cuttings. While, the effect of soaking time in yeast concentrations was not significant on studied parameters. However, the interaction effect of 2 g.L^{-} yeast with 2 followed 1 hour of soaking time gave the best results for most studied parameters.

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